Abstract: Increased plasma non-esterified fatty acids (FA) link obesity with insulin resistance (IR) and type 2 diabetes mellitus (T2DM). However, in contrast to the SFA acid palmitic acid, the MUFA oleic acid elicits beneficial effects on insulin sensitivity. Indeed, the dietary palmitic acid/oleic acid ratio impacts diabetic risk in humans. Here, we review the beneficial effects of oleic acid compared to palmitic acid on IR and T2DM, including its anti-inflammatory actions, and its capacity to inhibit endoplasmic reticulum stress, to prevent attenuation of the insulin signaling pathway and to improve beta-cell survival. Understanding the molecular mechanisms of the antidiabetic effects of oleic acid may contribute to understand the benefits of this FA in the prevention or delay of T2DM.
PALMITIC AND OLEIC ACID: THE YIN AND YANG OF FATTY ACIDS IN TYPE 2 DIABETES MELLITUS

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Abstract

Increased plasma non-esterified fatty acids link obesity with insulin resistance and type 2 diabetes mellitus. However, in contrast to the saturated fatty acid palmitic acid, the monounsaturated fatty acid oleic acid elicits beneficial effects on insulin sensitivity. Indeed, the dietary palmitic acid/oleic acid ratio impacts diabetic risk in humans. Here, we review recent mechanistic insights into the beneficial effects of oleic acid compared to palmitic acid on insulin resistance and type 2 diabetes mellitus, including its anti-inflammatory actions, and its capacity to inhibit endoplasmic reticulum stress, to prevent attenuation of the insulin signaling pathway and to improve β-cell survival. Understanding the molecular mechanisms of the antidiabetic effects of oleic acid may contribute to understand the benefits of this fatty acid in the prevention or delay of type 2 diabetes mellitus.
Increased plasma non-esterified fatty acids link obesity and insulin resistance

Insulin resistance, defined as a defect in the capacity of insulin to drive glucose into its target tissues, both predicts and precedes the development of type 2 diabetes mellitus (T2DM) [1]. This condition eventually leads to hyperglycemia when pancreatic β cells fail to secrete sufficient insulin to meet the increased metabolic demand for this hormone. The expansion of adipose tissue in obese individuals releases increased amounts of non-esterified fatty acids (NEFA) (see Glossary, also called free fatty acids, FFA), hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. Increased levels of NEFA provoke insulin resistance and impair β-cell function [2], making them good candidates as a link between obesity and T2DM development. The link between obesity and T2DM also involves the activation of a chronic low-level inflammatory process promoted by increased levels of NEFA, which contributes to insulin resistance and T2DM [1].

Plasma NEFA released by adipose tissue reflects fat intake. Oleic acid (C18:1) and palmitic acid (C16:0) are the most abundant dietary and plasma FA, and represent 31% and 27% respectively of total plasma NEFA [3]. However, the saturated FA (SFA) (Box 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differently contribute to insulin resistance. Studies of subjects who reduced their SFA and increased their MUFA intake have shown a significant improvement in insulin sensitivity [4]. This beneficial effect of oleic acid on insulin sensitivity can also help to explain the protective effects of the Mediterranean diet against both obesity and T2DM. That diet is characterized by a specific FA pattern with a low intake of SFA (7-8% of energy) and a high intake of MUFA (over 20% of total energy), as the primary fat source is olive oil (100 g contain 74 g of oleic acid) [5]. Understanding the molecular mechanisms
responsible for the beneficial effects of oleic acid might help promote its use in the prevention or delay of T2DM. Here, we review recent mechanistic insights into the beneficial effects of oleic acid compared to palmitic acid in insulin resistance and T2DM.

**Molecular mechanisms involved in palmitic acid-induced insulin resistance**

Three main mechanisms have been reported in palmitic acid-mediated insulin resistance and T2DM (Figure 1): 1) Increased synthesis of deleterious complex lipids; 2) Impaired function of cellular organelles; 3) Receptor-mediated inflammation. First, the increase in plasma NEFA favors the rate of FA delivery to non-adipose tissues, such as the liver, muscle, heart, and pancreatic islets. Such ectopic lipid deposition promotes metabolically relevant cellular dysfunction (lipotoxicity) and programmed cell-death (lipoapoptosis). In these circumstances, increased intracellular levels of palmitic acid exceed its mitochondrial oxidation and it is converted into deleterious complex FA-derived lipids, such as diacylglycerol (DAG) and ceramide. In contrast, the accumulation of the relatively inert triacylglycerol (TAG), which is not a signaling lipid, is generally considered to be harmless and it could even be protective [6]. The increase in the intracellular content of DAG caused by the exposure to enhanced levels of palmitic acid activates novel protein kinases C (PKC) isoforms, such as PKC0 in skeletal muscle and PKCε in liver [7], which ultimately attenuate the insulin signaling pathway by phosphorylating serine residues in insulin receptor substrate 1 (IRS-1). The activation of some of these PKC isoforms, such as PKC0, can also activate the pro-inflammatory signaling cascade IκB-kinase (IKK)β-IκB-nuclear factor (NF)-κB [7]; a pathway which also attenuates insulin action through IKKB-mediated phosphorylation
at serine residues of IRS-1 or by increasing the levels of pro-inflammatory cytokines. Interestingly, it has been reported that only DAG containing SFA in skeletal muscle membranes are related to insulin resistance in humans [8]. Likewise, the co-enzyme A thioester of palmitic acid is the limiting substrate of de novo ceramide synthesis. Therefore, the flux through this biosynthetic pathway is increased following enhanced availability of palmitic acid [9]. Increased ceramide synthesis leads to insulin resistance through several mechanisms, including inhibition of Akt via PKCζ and protein phosphatase 2A (PP2A), which promotes dephosphorylation and inactivation of Akt [9]. In addition, ceramide also induces endoplasmic reticulum (ER) stress, inhibits mitochondrial β-oxidation of FA and activates the nod-like receptor containing a pyrin domain (NLRP3) inflammasome [9]; a major instigator of inflammation.

Second, the mechanisms underlying the detrimental effects of excess NEFA on T2DM also include the impairment of the function of cellular organelles. Enhanced NEFA levels perturb ER homeostasis by causing lipid dysregulation in the ER that affects the lipid composition of the membranes of this organelle. This results in a process known as ER stress (Figure 1), which affects calcium signaling, can cause cell death, and attenuates protein translation [10]. In addition, palmitic acid-induced ER stress intersects with the inflammatory process by activating several pro-inflammatory pathways, such as NF-κB, c-Jun N-terminal kinase (JNK), double-stranded RNA (dsRNA)-dependent protein kinase (PKR) and the NLRP3 inflammasome [10]. Ultimately, all these changes contribute to metabolic dysregulation and T2DM [10]. Mitochondrial dysfunction has also been associated with muscle insulin resistance in several studies [11]. T2DM is characterized by the presence of disturbances in FA
metabolism, and mitochondria dysfunction can result in a reduced oxidative capacity promoting intracellular accumulation of complex lipids that activate pathways that interfere with the insulin signaling pathway. In addition, it has been proposed that increased lipid oversupply into the mitochondria because of increased NEFA levels leads to incomplete FA oxidation provoking increased generation of reactive oxygen species (ROS), with concomitant mitochondrial stress, which ultimately leads to cellular damage and insulin resistance [12]. Moreover, FA translocase, or CD36, is a scavenger receptor that recognizes long-chain FA and is involved in their trafficking; it also plays an important role in T2DM. In fact, palmitic acid is a potent inducer of ROS in several cell types, including β-cells, and CD36 appears to be required for FA-induced ROS production [13].

Third, palmitic acid can activate pro-inflammatory pathways through membrane receptors, such as Toll-like receptor 4 (TLR4): a pattern recognition receptor that recognizes bacterial components, including lipopolysaccharide (LPS). SFA can also activate TLR4 through fetuin A: an adaptor protein that mediates the interaction between SFA and TLR4. Once activated, TLR4 subsequently promotes the activation of pro-inflammatory transcription factors such as NF-κB [14]. Moreover, SFA can modify gut microbiota leading to an increase in the levels of LPS after high fat intake, enhancing this natural TLR4 ligand [15]. In addition to TLR4, another molecule that integrates immune and nutritional signaling with inflammation and insulin action is PKR, which is activated by viral dsRNA as part of the mammalian immune response. It has been suggested that small nucleolar RNA (snoRNA), which accumulate in the
cytosol and act as critical mediators of lipotoxicity, interact and activate PKR in a palmitic acid-dependent manner [16,17].

As mentioned above, substituting palmitic acid by oleic acid, together with additional lifestyle changes, offers an opportunity to reverse or delay the deleterious metabolic effects of this SFA. In fact, increased consumption of olive oil, which is high in oleic acid and contains antioxidant compounds, was associated with a reduction in the risk of T2DM in US women [18]. Likewise, it has been reported that the dietary palmitic acid/oleic acid ratio impacts diabetic risk in women [19]. Overall, these and other reports suggest that oleic acid has a protective effect against insulin resistance and T2DM. In the following sections, we describe the molecular mechanisms by which oleic acid prevents palmitic acid-induced insulin resistance and T2DM.

Oleic acid-activated mechanisms that attenuate the increase in the synthesis of deleterious complex lipids caused by palmitic acid

Skeletal muscle is a key tissue that affects the metabolic state of the whole organism since it accounts for about 80% of insulin-stimulated glucose disposal and is the main organ for NEFA utilization [20]. Accumulation of deleterious complex lipids (DAG and ceramide) within muscle fibers is considered a key factor in the development of insulin resistance [1] and may occur because of increased FA uptake due to excess plasma NEFA and/or because of a reduced rate of mitochondrial FA oxidation [21]. We previously reported that exposure of myotubes to palmitic acid leads to enhanced DAG levels and the subsequent activation of the pro-inflammatory pathway PKC0-NF-κB. This causes both phosphorylation of IRS-1 at serine residues and inhibition of insulin-
stimulated Akt phosphorylation, thereby attenuating insulin signaling [22] (Figure 2).

However, exposure to oleic acid does not cause these changes and co-incubation of palmitic acid-exposed cells with a lower concentration of oleic acid reverses the effects of the latter. When we examined the incorporation of these FA into TAG and DAG, we observed that palmitic acid was mainly incorporated into DAG, whereas oleic acid was mainly incorporated into TAG. Furthermore, cells incubated with both palmitic and oleic acid showed a greater incorporation of FA into TAG than cells exposed only to palmitic acid. These findings have been confirmed in additional studies [23]. The increased channeling of palmitic acid into DAG was associated with a reduction in the expression levels of diacylglycerol acyltransferase-2 (Dgat2), which is the enzyme that catalyzes the conversion of DAG into TAG; whereas no changes were observed in cells incubated with either oleic acid or palmitic plus oleic acid. These differences might explain why palmitic acid increases the levels of DAG and activates the PKC0-NF-κB pathway, whereas the addition of oleic acid favors the synthesis of TAG, protecting the cells from inflammation and the attenuation of the insulin signaling pathway. Moreover, palmitic acid and oleic acid affect the expression of genes involved in mitochondrial FA oxidation differently. The expression of these genes is regulated at the transcriptional level by several nuclear receptors, including PPARα and PPARβ/δ, whose potency as transcription factors largely depends on the assembly of transcriptional co-activators, such as PPARγ co-activator (PGC)-1α, which also controls mitochondrial biogenesis [24]. Palmitic acid reduces the expression of Pgc-1α in myotubes, whereas oleic acid does not, and co-incubation of palmitic acid-exposed cells with lower concentration of oleic acid prevents the reduction in its expression. The capacity of oleic acid to prevent the downregulation in the expression of Pgc-1α was dependent on PPARα and protein
kinase A (PKA) [22]. PGC-1α activity is also controlled through reversible acetylation. PGC-1α is deacetylated and activated by sirtuin 1 (Sirt1), and acetylated and inhibited by general control of amino-acid synthesis 5-like 2 (GCN5). Interestingly, Sirt1 deacetylase activity is induced by different signaling pathways, including AMPK and cyclic adenosine monophosphate (cAMP)/PKA, ultimately enhancing mitochondrial FA oxidation [25]. Importantly, myotubes exposed to oleic acid, but not to palmitic acid, display an increase in the levels of cAMP, which activate PKA. This results in SIRT1 phosphorylation at serine 434, which increases its deacetylase activity, eventually leading to enhanced activity of PGC-1α and expression of genes involved in mitochondrial FA oxidation, as well as the subsequent higher rate of FA oxidation [26]. Oleic acid also increases the levels of carnitine palmitoyltransferase 1 (CPT-1) [22,27], which enables the transport of FA into mitochondria for β-oxidation. This effect of oleic acid results in a reduction of ceramide and DAG levels in palmitic acid-exposed cells [27]. Further, co-incubation with oleic acid increases mitochondrial β-oxidation of the FA through a second mechanism: activation of AMPK, which in turn inhibits acetyl-CoA carboxylase (ACC) and consequently its product malonyl-CoA, a CPT-1 inhibitor [26]. Similarly, oleic acid abolishes palmitic acid-induced ceramide synthesis in myotubes by preventing the upregulation of dihydroceramide desaturase 1: the enzyme responsible for generating ceramide from its precursor. This effect of oleic acid protects cells from attenuation of the insulin signaling pathway caused by the SFA [28].

In the liver, an excess of SFA, such as palmitic acid, induces lipotoxicity, leading to the development of non-alcoholic fatty liver disease (NAFLD), a process associated with insulin resistance. Moreover, the severity of NAFLD correlates with the presence of lipoapoptosis. In cultured hepatocytes, palmitic acid, but not oleic acid, impairs the
insulin signaling pathway [29]. Likewise, co-incubation with both FA increases the extent of steatosis, but oleic acid reduces apoptosis and the impairment of insulin signaling caused by palmitic acid. Moreover, palmitic acid-induced apoptosis suppresses autophagy by inducing caspase-dependent beclin 1 cleavage. In contrast, it was suggested that oleic acid protects hepatocytes against apoptosis by inducing the formation of TAG-enriched lipid droplets and induction of autophagy [30].

On the other hand, chronic exposure of rodent and human islet β cells to FA impairs glucose-stimulated insulin secretion (GSIS) and induces apoptosis through the activation of several pathways, including increased synthesis of ceramide, mitochondrial dysfunction, ER stress and activation of the long-chain FFA receptor 1 (FFAR1) or G protein-coupled receptor 40 (GPR40) [31,32]. As for other cell types, oleic acid protects palmitic acid-exposed β cells, modifying the metabolism of the SFA either by increasing its oxidation or promoting its incorporation into TAG, thereby reducing the levels of ceramide and DAG, or by preventing ER stress [33-35]. The increase in palmitic acid-induced mitochondrial oxidation caused by oleic acid is mediated through PPARβ/δ activation [36].

**Oleic acid-activated mechanisms that prevent impaired function of cellular organelles caused by palmitic acid**

ER stress is a potentially new mechanism involved in the association between saturated NEFA-induced inflammation and insulin resistance [10]. We [37] and others [23] have reported that palmitic acid increases ER stress and inflammation and attenuates the insulin signaling pathway in mouse and human myotubes and in vivo. Oleic acid, in contrast, does not, and co-incubation of palmitic acid-exposed cells with oleic acid in a
lower concentration than the palmitic acid prevents these changes (Figure 3). This beneficial effect of oleic acid on palmitic acid-exposed cells is dependent on the AMP-mediated activation of AMPK [37], a metabolic sensor that also modulates inflammation and is recognized as a therapeutic target for diabetes [38]. The reduction in the levels of phosphorylated AMPK caused by palmitic acid in myotubes has also been associated with an increase in the levels of phosphorylated ribosomal protein S6 kinase 1 (S6K1), a downstream kinase of mammalian target of rapamycin (mTOR) that attenuates the insulin signaling pathway by phosphorylating IRS-1 at serine residues [39]. Oleic acid does not affect the phosphorylation of S6K1, and co-incubation of palmitic acid-exposed myotubes with oleic acid prevents the increase caused by the SFA. In another study in human and mouse hepatocytes, oleic acid protected against the deleterious effects of palmitic acid on ER stress, apoptosis and the insulin signaling pathway by preventing S6K1 activation [40]. In a similar way, co-incubation of palmitic acid-exposed β cells with oleic acid protects the cells by activating pro-survival pathways of the ER stress response involved in protein folding and antioxidative defense [41].

Tribbles 3 (TRB3) is a pseudokinase, which contains a kinase domain without enzymatic activity and is responsible for strikingly different metabolic functions, depending on the tissue studied. In the liver, TRB3 binds to Akt and inhibits its activity, leading to impaired insulin signaling [42]. In hepatocytes, TRB3 upregulation occurs through palmitic acid-induced ER stress and the increased levels of this protein are sufficient to modulate FA-induced insulin resistance [43]. Oleic acid does not increase
TRB3 levels and co-incubation of palmitic acid-exposed cells with oleic acid prevents the increase in the levels of this protein.

Mitochondria are a major producer of ROS and exposure of skeletal muscle cells to palmitic acid causes a significant increase of mitochondrial ROS production and a concomitant mitochondrial DNA damage and dysfunction, induction of JNK, apoptosis, and inhibition of insulin signaling [44]. In contrast, supplementation with oleic acid reduces ROS generation and protects the mitochondria from palmitic acid-induced oxidative stress, apoptosis and attenuation of insulin signaling [44,45].

**Oleic acid-mediated mechanisms that prevent palmitic acid-induced inflammation**

IL-1β is a potent cytokine that plays a key role in inflammation and insulin resistance [46]. In fact, lack of IL-1β signaling results in improved insulin sensitivity in HFD-fed mice [47]. The release of this potent cytokine requires two steps (Figure 4). First, activation of the TLR4/NF-κB pathway by palmitic acid leads to the production of pro-IL-1β. Second, the NLRP3 inflammasome generates mature IL-1β through caspase-1-dependent processing activated by several molecules, including ATP. It should be noted that substitution of SFA by an oleic acid-enriched diet in HFD-fed mice results in improved insulin sensitivity, reduced pro-IL-1β levels and adipose IL-1β secretion and sustained adipose AMPK activation [48]. Furthermore, oleic acid prevents ATP-induced IL-1β secretion from LPS- and palmitic acid-exposed macrophages through an AMPK-dependent mechanism. The findings of this study indicate that sustained activation of
AMPK by oleic acid disrupts NLRP3 inflammasome activation, reducing the processing of IL-1β and the attenuation of insulin signaling caused by this cytokine.

Several factors can increase the diabetogenic effect of visceral adipose tissue, including increased production of adipokines, which can impair insulin sensitivity. This production of cytokines is enhanced by the infiltration of macrophages into visceral adipose tissue [49]. Macrophages undergo specific differentiation depending on the local tissue environment, to fulfill their functionally distinct roles, and they are capable of polarizing toward different phenotypes, which include the classical (pro-inflammatory, M1) and alternative (anti-inflammatory, M2) activation states. In obesity, adipose tissue M1 macrophage numbers increase and correlate with adipose tissue inflammation and insulin resistance; whereas in lean humans and mice, M2 macrophages predominate [50].

The effects of different FA have been evaluated in mature visceral adipocytes from non-obese and morbidly obese subjects [51]. Palmitic acid shows a direct inflammatory effect in adipocytes from non-obese subjects, increasing the expression of the pro-inflammatory cytokines TNF-α and IL-6 and decreasing the mRNA levels of the anti-inflammatory cytokine IL-10 and adiponectin: the most abundant peptide secreted by adipocytes, reduction of which plays a central role in insulin resistance/T2DM. These pro-inflammatory effects of palmitic acid are more pronounced in adipocytes from morbidly obese patients. In contrast, oleic acid decreases the expression of pro-inflammatory cytokines and causes a pronounced increase in IL-10 and adiponectin expression in adipocytes from non-obese patients (Figure 4). Interestingly, adiponectin increases cellular ceramidase activity, which catalyzes ceramide degradation and activates AMPK, which in turn can activate FA oxidation [52]. The anti-inflammatory
effect of oleic acid was abolished by knocking down the expression of FFAR4/GPR120, a receptor with anti-inflammatory effects that is activated by long-chain FA, mainly n-3 PUFA, but also by oleic acid [53]. It is noteworthy that the pro-inflammatory effect of palmitic acid was also reversed by silencing FFAR4/GPR120, although no explanation was provided for this finding. Interestingly, the anti-inflammatory effect of oleic acid was not observed in adipocytes from morbidly obese subjects [51]. This might be a consequence of the reported reduced expression of FFAR4/GPR120 in visceral adipose tissue of morbidly obese patients [54]. Consistent with FFAR4/GPR120 playing a role in the mediation of the effects of oleic acid, an oleic acid-enriched diet improved whole-body insulin resistance in an animal model of diet-induced obesity by: reducing inflammation in adipose tissue, liver and skeletal muscle; decreasing macrophage infiltration; and increasing IL-10 levels, and all these effects were abolished by FFAR4/GPR120 knockdown [55]. Additionally, oleic acid increases M2 macrophage markers in mesenteric adipose tissue, suggesting that this MUFA induces M2 macrophage polarization [56].

Oleic and palmitic acid also elicit opposite effects on macrophages/Kupffer cells and hepatocytes [57]. In fact, a conditioned medium from macrophages stimulated with palmitic acid induces ER stress and inflammatory signaling that impairs insulin signaling in mouse hepatocytes. In contrast, oleic acid-stimulated macrophages result in M2 polarization and the conditioned medium from these cells enhances insulin sensitivity in hepatocytes. This is due to a reduction in the levels of leukotriene B₄, which in turn leads to a reduction in hepatocytes of both protein tyrosine phosphatase 1B (PTP1B), and phosphatase and tensin homolog (PTEN): two phosphatases that negatively modulate insulin receptors and Akt, respectively. Oleic acid also upregulates
microRNA-21, inducing PTEN degradation; whereas palmitic acid has no effect [58]. Moreover, oleic acid increases the expression of PPARβ/δ in human hepatocytes via a FFAR1/GPR40-mediated and calcium-dependent mechanism. The increase in PPARβ/δ caused by oleic acid then negatively regulates PTEN and thereby increases insulin sensitivity [59].

Attenuation of insulin-induced tyrosine phosphorylation and methylation of the catalytic subunit of PP2A are involved in the activation of this enzyme by palmitic acid, and ultimately result in the repression of Akt activation/phosphorylation. In rat and human skeletal myotubes the effect of palmitic acid on PP2A is antagonized by oleic acid through increased tyrosine phosphorylation and demethylation of the catalytic subunit of PP2A [60], which results in the repression of the phosphatase activity of this enzyme.

**Additional antidiabetic effects of oleic acid**

Part of the effects resulting from dietary intake of oleic acid may be mediated by its derived endogenous lipid mediator oleoylethanolamide (OEA). Dietary intake of oleic acid elevates the levels of OEA by increasing substrate availability for its biosynthesis from membrane glycerophospholipids [61]. Interestingly, OEA decreases food intake and body weight gain through peroxisome proliferator-activated receptor (PPAR)α activation. The reduction in body weight caused by OEA has been attributed to its anorectic activity as well as to its capacity to increase lipolysis in adipocytes and the subsequent stimulation of FA oxidation in skeletal muscle [61]. Additionally, oleic acid ingestion stimulates OEA mobilization into the mucosal cells of the gut, which activates
a PPARα-mediated signal that travels through the afferent vagus nerve to the hypothalamus, where it increases satiety [62].

On the other hand, dietary fat is known to stimulate the release by intestinal L-cells of glucagon-like peptide 1 (GLP-1), which in turn increases GSIS, among other physiological effects. The release of GLP-1 by dietary fat is mediated by NEFA through FFAR1/GPR40. In addition, oleic acid can also induce GLP-1 secretion in vivo via PKCζ [63]. Meanwhile, GPR119 is an antidiabetic target expressed in β cells and in incretin-secreting enteroendocrine cells in the intestine [64]. Activation of GPR119 can increase insulin secretion directly by acting at the level of the β-cell or indirectly by increasing circulating levels of GLP-1 released from intestinal L-cells. Of all the endogenous GPR119 agonists, OEA is the more potent [64]. There are three additional oleic acid derivatives that are GPR119 agonists [64]. However, there are conflicting reports regarding whether these compounds are responsible for fat-induced GPR119 activation and GLP-1 release [64]. OEA can also elicit a powerful cytoprotective effect on palmitic acid-exposed rat β cells. However, this effect is not mediated by GPR119, since OEA was internalized and subjected to hydrolysis by FA amide hydrolase to release free oleic acid, which in turn mediated cytoprotection [65].

Some of the effects of oleic acid might also be mediated by its effects on the hypothalamus, a region that regulates energy homeostasis. The hypothalamus matches energy intake to energy expenditure to prevent obesity through satiety (leptin) and hormonal (insulin) signals. In addition, the hypothalamus interacts with the liver through the vagus nerve. On sensing FA, the hypothalamus lowers food intake and
glucose production [66]. For instance, central oleic infusion inhibits glucose production and food intake [67]. Exposure to palmitic acid, but not oleic acid, by oral gavage or direct infusion results in PKC0 activation in the hypothalamus, which was associated with impaired hypothalamic insulin and leptin signaling [68]. In humans, increased levels of oleic acid in cerebrospinal fluid were associated with improved glucose tolerance [69].

Cardiovascular disease is a major cause of morbidity and mortality in T2DM patients, and the presence in these patients of atherogenic dyslipidemia is among the major factors contributing to increased cardiovascular risk. It is now generally accepted that the different components of atherogenic dyslipidemia are closely linked and are initiated by insulin resistance through overproduction of TAG-rich very-low-density lipoproteins (VLDL) [70]. Interestingly, infusion of oleic acid in the hypothalamus of rats activates a neuronal network that suppresses VLDL-TAG secretion in the liver [71]. Oleic acid also shows a beneficial effect at the cardiovascular level, compared with palmitic acid [72].

Concluding remarks and future perspectives

There is compelling evidence that insulin resistance and T2DM can be prevented or their onset delayed by lifestyle interventions; and it is critical to promote changes in diet that reduce the incidence of T2DM [73]. It is now well accepted that the total amount of fat intake has an impact on insulin sensitivity only if it exceeds the 37% of total energy intake [4]. However, below this value, a critical factor in the induction of insulin resistance is not the total amount of fat, but its FA composition [4]. In fact, insights gained from preclinical studies have shown that substitution of part of the palmitic acid by oleic acid strongly attenuates the deleterious effects of the SFA on adipose tissue,
skeletal muscle, liver and β cells. Consistent with this, substituting dietary SFA for a high-oleic-acid diet enhances insulin sensitivity in humans [4]. Several mechanisms account for the protective effects of oleic acid with respect to T2DM. However, the studies have some limitations and many questions remain regarding the effects of oleic acid on humans (see Outstanding Questions). Interestingly, oleic acid activates or prevents the reduction in AMPK activity caused by palmitic acid. AMPK is a recognized therapeutic target for T2DM [38] and it is also the main target activated by metformin, the most commonly prescribed drug for T2DM in the world [38]. Some studies have demonstrated that oleic acid and metformin have similar protective effects, both preventing the deleterious effects of palmitic acid [39]; this suggests that oleic acid might have some metformin-like effects. Current and future studies should elucidate the mechanisms responsible for the protective effects of oleic acid against T2DM development in humans.
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FIGURE LEGENDS

Figure 1. Palmitic acid attenuates the insulin signaling pathway through different mechanisms leading to insulin resistance.

As visceral obesity develops, it results in greater release of NEFA, since visceral adipocytes are more sensitive to lipolytic stimuli. In addition, insulin resistance leads to the failure of lipolysis inhibition by this hormone and further augments the increase in plasma NEFA levels. The increase in plasma NEFA, mainly the saturated palmitic acid, results in inflammation and insulin resistance via three main mechanisms. 1. Increased internalization of palmitic acid results in lipotoxicity when this FA exceeds oxidative needs and spills over into deleterious non-oxidative metabolic pathways, increasing the levels of diacylglycerol (DAG) and ceramide. DAG activates PKC, which in turn attenuates the insulin signaling pathway by phosphorylating IRS-1 at serine residues and it also activates the IKKβ-NF-κB pathway, further exacerbating the impairment of insulin signaling and provoking inflammation. The increase in ceramide activates the NLRP3 inflammasome-mediated release of IL-1β, and it also leads to activation of PP2A and PKC, which both attenuate the insulin signaling pathway. 2. The excess of palmitic acid impairs the function of the ER and mitochondria. Impairment of ER homeostasis results in ER stress that promotes inflammation (NF-κB, JNK and NLRP3 inflammasome pathways are activated) and apoptosis (e.g. β cells). The impairment of mitochondrial function may reduce FA oxidation and it can increase ROS generation. 3. Palmitic acid can activate TLR-4 through fetuin A and high-fat diets increase the levels of LPS, an activator of this receptor, leading to an increase in the activity of the IKKβ-NF-κB pathway.
Akt: mammalian homologue of retroviral oncogene v or protein kinase B; DAG: diacylglycerol; ER: endoplasmic reticulum; FA: fatty acids; FAT: fatty acid translocase; FATP: fatty acid transporter protein; IL: interleukin; IκB: inhibitor of κB; IKKβ: inhibitor of nuclear factor (NF)-κB kinase subunit β; IRS: insulin receptor substrate; JNK: c-Jun N-terminal kinase; LPS: lipopolysaccharide; NLRP3: nod-like receptor containing a pyrin domain; NF-κB: nuclear factor-κB; PI3K: phosphatidylinositol 3 kinase; PKC: protein kinase C; pS: phosphorylated serine; PP2A: protein phosphatase 2A; pY: phosphorylated tyrosine; p50/p65: NF-κB subunits; ROS: reactive oxygen species; TAG: triacylglycerol; TLR4, Toll-like receptor 4.

Figure 2. Oleic acid prevents the palmitic acid-induced increase in the synthesis of deleterious complex lipids. Oleic acid prevents the deleterious effects of palmitic acid by increasing the mitochondrial oxidation of the SFA and by promoting their accumulation in the form of TAG, thereby reducing the synthesis of DAG and ceramide. The increase in FA oxidation caused by oleic acid is mediated by the restoration of AMPK activity and the increase in the expression of Cpt-1 and Pgc-1α. In addition, oleic acid increases the activity of PGC-1α through the activation of Sirt1 and the subsequent reduction of the acetylated (inactive) form of this protein. Oleic acid prevents the reduction in AMPK activity caused by palmitic acid and ultimately inhibits ER stress and inflammation as well as the activation of the mTORC1-S6K1 pathway. Blue arrows show the potential effects of oleic acid.
Ac: acetylated; Akt: mammalian homologue of retroviral oncogene v or protein kinase B; AMPK: AMP-activated protein kinase; CPT-1: carnitine palmitoyl transferase 1; DAG: diacylglycerol; DAGT2: diacylglycerol acyltransferase 2; FA: fatty acids; FAT: fatty acid translocase; FATP: fatty acid transporter protein; IκB: inhibitor of κ B; IKKβ: inhibitor of nuclear factor (NF)κ-B kinase subunit β; IL: interleukin; IRS: insulin receptor substrate; NF-κB: nuclear factor-κB; p50/p65: NF-κB subunits; PGC-1α: PPARγ co-activator 1α; PI3K: phosphatidylinositol 3 kinase; PKC: protein kinase C; PPAR: peroxisome proliferator-activated receptor; pS: phosphorylated serine; PP2A: protein phosphatase 2A; pY: phosphorylated tyrosine; p50/p65: NF-κB subunits; ROS: reactive oxygen species; RXR: retinoid X receptor; SFA, saturated fatty acids; TAG: triacylglycerol.

**Figure 3. Oleic acid prevents the impairment in the function of cellular organelles caused by palmitic acid.** Oleic acid prevents the reduction in AMPK activity caused by palmitic acid and ultimately inhibits ER stress and inflammation as well as the activation of the mTORC1-S6K1 pathway. Oleic acid also prevents the increase in the levels of the ER stress-induced pseudokinase TRB3 caused by palmitic acid. Moreover, oleic acid attenuates ROS generation and protects the mitochondria from palmitic acid-induced oxidative stress.

Akt: mammalian homologue of retroviral oncogene v or protein kinase B; AMPK: AMP-activated protein kinase; ER, endoplasmic reticulum; FAT: fatty acid translocase; FATP: fatty acid transporter protein; IκB: inhibitor of κ B; IKKβ: inhibitor of nuclear
factor (NF-κB) kinase subunit β; IL: interleukin; IRS: insulin receptor substrate; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor-κB; p50/p65: NF-κB subunits; PI3K: phosphatidylinositol 3 kinase; pS: phosphorylated serine; pY: phosphorylated tyrosine; p50/p65: NF-κB subunits; ROS: reactive oxygen species; TNF-α: Tumour necrosis factor α.

**Figure 4. Oleic acid prevents palmitic acid-induced inflammation.**

Oleic acid elicits anti-inflammatory effects by reducing the levels of cytokines (IL-6 and TNF-α), while increasing the levels of the anti-inflammatory cytokine IL-10 and adiponectin. These effects of oleic acid might be mediated by FFAR4/GPR120. The increase in the levels of adiponectin caused by oleic acid can activate AMPK and reduce the levels of palmitic acid-induced ceramide synthesis. By preventing the reduction in AMPK caused by palmitic acid, oleic acid can inhibit the increase in IL-1β caused the SFA through NLRP3 inflammasome in adipocytes and macrophages that infiltrate adipose tissue. Oleic acid might also favor the polarization of adipose tissue-infiltrated macrophages to the M2 anti-inflammatory phenotype. Oleic acid induces polarization of macrophages to the M2 anti-inflammatory phenotype, reducing the secretion of LTB4, which in turn decreases the activity of the phosphatases PTEN and PTP1B, thereby enhancing insulin sensitivity.

Akt: mammalian homologue of retroviral oncogene v or protein kinase B; AMPK, AMP-activated protein kinase; IL: interleukin; FA: fatty acids; GPR120/FFAR4: G-
protein-coupled receptor 120/free fatty acid receptor 1; IRS: insulin receptor substrate; LTB4: leukotriene B4; NF-κB: nuclear factor-κB; NLRP3: nod-like receptor containing a pyrin domain; PIP2: phosphatidylinositol 2-phosphate; PIP3: phosphatidylinositol 3-phosphate; PI3K: phosphatidylinositol 3 kinase; PTEN: phosphatase and tensin homolog; PTP1B: protein tyrosine phosphatase 1B; pY: phosphorylated tyrosine; p50/p65: NF-κB subunits; SFA, saturated fatty acid; TLR4, toll-like receptor 4.

**BOX 1: Fatty acids and the oleic acid-derivative oleoylethanolamide.**

It is recommended that dietary fat constitute 20-35% of energy intake [74]. However, the type of FA present in the fat ingested is of considerable importance in the development of insulin resistance/T2DM. FA can be classified according to their chain length into: short-chain FA, with 2-6 carbon atoms; medium-chain FA, with 7-12 carbon atoms; long-chain FA, with 13-22 carbon atoms; and very-long-chain FA, with more than 22 carbon atoms. They are also divided into saturated FA (SFA), with no carbon-carbon double bonds; monounsaturated FA (MUFA), with a single double bond (alkene) in the FA chain; and polyunsaturated FA (PUFA), containing several double bounds. PUFA are subdivided into omega-6 (n-6) and omega-3 (n-3) acids depending on the distance from the first double bond to the methyl end. The ingestion of oleic acid can stimulate the production of oleoylethanolamide (OEA) in enterocytes [62]. However, excess fat can reduce the synthesis of OEA. OEA belongs to the group of FA called ethanolamides or N-acylethanolamines, which are a class of naturally occurring bioactive signaling lipids derived from SFA and unsaturated FA. OEA controls food intake and promotes fat catabolism. Fat ingestion results in the release of oleic acid in the small-intestinal lumen, which is internalized by the enterocytes and channeled
toward the synthesis of chylomicrons or OEA. The latter can then be hydrolyzed into oleic acid and ethanolamine by FA amide hydrolase (FAAH).
Substituting saturated fatty acids by oleic acid in the diet improves insulin sensitivity in humans.

Preclinical studies have shed light on the molecular mechanisms by which oleic acid prevents palmitic acid-induced inflammation and insulin resistance in adipose tissue, liver, skeletal muscle, and pancreas.

The hypothalamus also senses oleic acid, where it activates a neuronal network that suppresses VLDL-TAG secretion in liver.

Oleic acid protects against cardiovascular insulin resistance and the atherosclerotic process.

Some of the effects of oleic acid are mediated by preventing the reduction in palmitic acid-mediated AMPK activity, resembling the action of metformin.
Outstanding Questions

Which oleic acid-activated molecular mechanism is the most important for preventing palmitic acid-induced insulin resistance?

Are the molecular mechanisms activated by oleic acid reported in preclinical models also observed in humans?

How much does OEA contribute to the effects of oleic acid? Are the effects of OEA on the release of GLP-1 mediated by GPR119 activation or are other molecular mechanisms involved?

Could an oleic acid-enriched diet be considered a non-pharmacological treatment to increase AMPK activity in humans, especially in those populations with a low consumption of this MUFA?

What is the contribution of oleic acid consumption to the beneficial effects of the Mediterranean diet on the prevention of insulin resistance and T2DM?
Figure 1

1. Increased levels of deleterious complex lipids
2. Impaired function of cellular organelles
3. Receptor-mediated inflammation

Visceral obesity → Adipose tissue lipolysis → Palmitic acid → TLR4

Fetuin A → MYD88 → NLRP3

FAT/CD36 → FATP → DAG → Ceramide → FA β-oxidation → CPT-1

CPT-1 → ROS → ER stress

TLR4 → IKKβ → IκB → p50, p65

NF-κB → IL-6, TNF-α, pro IL-1β, .... → Cytokines → Insulin resistance

Insulin receptor → IRS → PI3K → Akt → GLUT4

High-fat diet → LPS
Figure 2

Palmitic acid

FAT/CD36

FATP

Insulin receptor

Insulin

Insulin signaling pathway

Cytokines

Insulin resistance

IRS

pY

pS

pS

Akt

CPT-1

TAG

DGAT2

IκB

p50

p65

NF-κB

IL-6, TNF-α

PP2A

PPAR

PGC-1α

PGC-1α

RXR

CPT-1

Ac

Cytokines

Glucose

FATP

Oleic acid

Bioenergetics

Palmitic acid, Oleic acid

AMPK

p

Sirt1

PGC-1α

Ac

CPT-1

FA oxidation

DGAT2

TAG

Ceramide

IκB

p50

p65

NF-κB

Cytokines

Insulin resistance

Palmitic acid, Oleic acid

AMPK

Sirt1

PGC-1α

Ac

CPT-1

FA oxidation

DGAT2

TAG

Ceramide

IκB

p50

p65

NF-κB

Cytokines

Insulin resistance
Figure 3

Palmitic acid

FAT/CD36

FATP

Oleic acid

AMPK

mTOR

S6K1

IRS

PI3K

Akt

TRB3

Glucose

GLUT4

Insulin receptor

Insulin signaling pathway

CPT-1

ROS

NF-κB

p50

p65

IKKβ

IKKβ

p50

p65

p50

p65

IL-6, TNF-α

Nucleus

Insulin resistance

Cytokines
Figure 4

- Oleic acid
- Palmitic acid
- FFAR4/GPR120
- TLR4
- MYD88
- CD14
- IL-6
- TNF-α
- IL-10
- Adiponectin
- Ceramide
- ADipoR1/R2
- AMPK
- Oleic acid
- Palmitic acid
- NLRP3
- IRS
- PTP1B
- PI3K
- PTEN
- LTB4
- PI3K
- GLUT4
- Glucose
- Insulin
- Insulin sensitivity
- M2 genes
- M2-like macrophage
- Anti-inflammatory effects
- β-arrestin
- IL-1β
- Pro-IL1β
- IL1β
- NF-κB
- p50
- p65
- Pro IL1-β
- Nucleus
- Insulin resistance
Figure I

- Palmitic acid (C16:0)
- Oleic acid (C18:1 n-9)
- Oeloylethanolamide (OEA)
November 22th, 2017

Matthew BEYMER
Editor, Trends in Endocrinology & Metabolism

TEM-D-17-00153

Dear Dr. Beymer,

According to your instructions we wish to submit a reworked version of our manuscript entitled “PALMITIC AND OLEIC ACID: THE YIN AND YANG IN T2DM”.

We submit a letter where we have addressed, point-by-point, all the issues raised by the reviewers. I thank you in advance for your interest in our work.

Yours truly,

Manuel Vázquez-Carrera
Editorial Comments

We are very grateful to the comments of the Editor (A nice timely review focusing the opposing effects of palmitate and oleate).

Comment 1. INTRODUCTION
(increase plasma…) Nice however rather lengthy I would limit the explanation of T2D and focus on setting up background on Oleic and Palmitic acids (see marked up manuscript).

According to the editor suggestion, the length of the introduction section has been reduced.

Comment 2. (molecular mechs involved in palmitic…..) A very nice thorough and detailed explanation of the molecular mechanisms of palmitate-induced Insulin Resistance. Currently, I would say this section is a bit too long however if you focus and edit the following sections as suggested I would say it is sufficient.

To reduce the length of the manuscript, we have deleted a paragraph in this section referring to the athlete paradox.

Comment 3. CONTENT/ STRUCTURE/WORDING AND ACCESSIBILITY
As noted by Reviewer 2 I believe that the manuscript can be greatly improved with some re-organization. It does seem that through the review you are discussing tissue-specific effects of palmitate and oleate. Given the length of the current manuscript perhaps it would be prudent to keep examples from each tissue as evidence for the actions of oleate or palmitate and move tissue-specific actions to text boxes instead if this is something you are in fact trying to highlight, this would, however, require the elimination of the current figures, but from an editorial perspective I believe the 4 text boxes (adipose, skeletal, hepatic, and beta-cell specific actions) and one solid figure on palmitate actions and one solid figure on basic oleate actions would be very beneficial to the reader.

We really appreciate the efforts of the editor and reviewer 2 to improve the manuscript. We have tried to follow this suggestion, but we found that describing the general mechanisms by which oleic acid prevents the deleterious effects of palmitic acid and showing additional examples in specific tissues in text boxes might be a little redundant. As an alternative, we decided to describe the effects of oleic acid on the three main mechanisms responsible for palmitic acid-induced insulin resistance.

Comment 4. TEXT BOXES
I would suggest creating a text box instead of a figure for each of the tissues and transferring tissue-specific effects to these leaving common effects (S6K1, AMPK, etc.) in the main text. I would then suggest taking the current text box and incorporating it into the main text.

Following the explanation provided in comment 3, we have modified the figures and we have decided to keep Text box I.

Comment 5. FIGURES AND FIGURE LEGENDS
The figures are good however a bit complicated to follow and a bit more complex than a typical TEM review figure.
**Figure 1:** I would suggest keeping this figure however in order to make it a bit more visually accessible it might be useful to switch the location of the Insulin receptor and TLR4 so that the effector arrows do not cross over each other as much.

**Figures 2-5:** as suggested above consider converting these to text boxes.

**Figure 1:** perhaps incorporate these into the figures for palmitate or oleate respectively.

We have simplified Figure 1. In addition, in the revised version of the figure the three main mechanisms activated by palmitic acid have been highlighted. We greatly appreciate the suggestion of the editor to make less complicated the figure.

Figures 2-4 have been modified and now reflect the preventive effects of oleic acid on the three main mechanisms by which palmitic acid promotes insulin resistance.

We have kept Figure 1.

**Comment 6. LENGTH**
At just over 4900 words the manuscript is significantly longer than we allow for a TEM review. Typically we allow between 3500 with some room for expansion. This means that the manuscript should be edited significantly as stated above.

We have reduced the length of the manuscript to 4197 words.

**Comment 7. CLARITY/ACCESSIBILITY**
Please review the changes I have suggested in the main text and make any modifications necessary if the intended scientific meaning has been altered.

We have accepted the suggested changes.
Comments for Reviewer 1

We would like to thank the reviewer for his/her useful suggestions, which have allowed us to include changes to improve the manuscript.

Specific Comments:

Comment 1. In page 8, the title next to "Oleic acid prevents palmitic acid-induced insulin resistance in metabolic tissues" seems not suitable to show "Adipose tissue". Please revise to easier way.

Following the suggestion of the editor and reviewer 2, we have re-organized the manuscript and in the revised version we describe the preventive effects of oleic acid on the three main mechanisms activated by palmitic acid: 1. Increased synthesis of deleterious complex lipids. 2. Impaired function of cellular organelles. 3. Receptor mediated inflammation.

Therefore, in the new version of the manuscript this heading no longer exists.

Comment 2. Skeletal muscle is a key metabolic tissue that affects the metabolic state of the whole organism, as you mentioned. But skeletal muscle was arranged not at the first section. Why?

The reviewer is right and in the revised version of the manuscript the main effects of oleic acid on skeletal muscle are shown in the first section.

Comment 3. Similar to the clear indications of palmitic acid-induced insulin resistance, it would be better to summarize the clear mechanisms for oleic acid prevents palmitic acid-induced insulin resistance.

We believe that the re-organization of the manuscript has improved this aspect (please, see comment 1).

Comment 4. Suitable dose for oleic acid or OEA and the damage dose of palmitic acid in human subjects were ignored. Why?

Usually, scientific associations make general assessments or recommendations about diet. For instance, the general recommendation for obesity and diabetes is to follow a low-fat diet (<30% of total energy). In addition, these scientific associations recommend replacing saturated with monounsaturated or polyunsaturated fat (please, see Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation 2017, in press; or reference 69 of the manuscript). In contrast, no doses are recommended.

Comment 5. In Figure 2, receptors for oleic acid were indicated as GPCR but it was different in Figure 3, 4 and 5. Please give the reason(s) in context.

These receptors have historically received two names. For instance, FFA receptor 1 (FFAR1) or G protein-coupled receptor 40 (GPR40). In the revised version of the manuscript we have included both names.

Comment 6. GPR120 was not showed in Figure 2 for binding with oleic acid. Why?
In the revised version of the manuscript this receptor appears in Figure 4 and it is bound to oleic acid.

**Comment 7.** A high similarity (66 %) with previous report (McAinch AJ, Cornell LM, Watts R, et al., Eur J Nutr (Germany), 2015, 54(7): 1033-1043) observed. Please check it. We did not use this reference for preparing the manuscript that describes the effects of fatty acids on PDK4 in cultured myotubes from obese and diabetic individuals. Any similarity is by chance.
Comments for Reviewer 2

We are very grateful to the comments of this reviewer. The topic is very important, and writing a review which summarizes the current knowledge in this field would be highly appreciated by researchers. Such review is also important in the context of our efforts to prevent or to delay the disease.

In addition, we would like to thank the reviewer for her/his useful suggestions, which have allowed us to include changes to improve the manuscript.

Major comments:

Comment 1. I acknowledge the complexity of writing a review on this topic. However, I believe that the review can be improved if the text is slightly re-organized and presented in a somewhat different way. In the current form, the review is not easy to follow and also the focus and the aim of the review is kind of blurred. I think that figures are also not optimal and not really facilitate the reading. Authors prepared 5 figures with schematic views of how fatty acids affect cells. I assume that the first figure is supposed to give an overall view of palmitate effects on the cells whereas figures 2-5 should reflect the tissue-specific protection by oleate.

However, the impression from the text and from the figures is that the intention of authors is to compare the mechanisms of fatty acid action on different cells. The review makes readers to think that some mechanisms are tissue-specific. However, I do not think that these messages are what authors aim to say.

The reviewer is right and we greatly appreciate his/her efforts to improve the manuscript. Following the suggestion of the reviewer and the editor, we have re-organized the manuscript. In the revised version of the manuscript we first describe the three main mechanisms by which palmitic acid increases insulin resistance (Figure 1) and then we describe the beneficial effects of oleic acid on these palmitic acid-activated mechanisms (Figures 2-4).

Comment 2. Some specific comments that the major comment is based on (something is missing here)

Title of the first section is "Increased plasma non-esterified fatty acids link obesity and insulin resistance". However, in the text they claim that "Increased levels of NEFA provoke insulin resistance and impair β-cell function, making them good candidates as a link between obesity and T2DM development". With this sentence authors link NEFA with beta-cell dysfunction. I think an information about the toxic effect of NEFA on beta cells would be relevant and helpful in this section.

The link between NEFA with beta-cell dysfunction is explained thoroughly later; please see page 10 (Chronic exposure of rodent and human islet β cells to FA impairs glucose-stimulated insulin secretion (GSIS) and induces apoptosis).

In our opinion, including information in this section about the toxic effects of NEFA on beta cells is a little bit premature. However, following the suggestion of the reviewer, we have included a reference of a review on the toxic effects of increased NEFA on beta cells to provide more information to the readers interested in this subject.

Comment 3. In the second section "Molecular mechanisms involved in palmitic acid-induced insulin resistance" authors write that "Three main mechanisms have been reported
in palmitic acid-mediated insulin resistance and T2DM”. However, it is not clear what authors mean by these three mechanisms. Those mechanisms need to be clarified at the beginning of the section. After reading of whole section my understanding is that these mechanisms are: 1) uptake of NEFA by non-adipose tissue, 2) impairment of cellular organelles, 3) activation of pro-inflammatory pathways through membrane receptors. Authors need to explain of how the effects of fatty acids have been classified into these three mechanisms. Especially, if ER stress and inflammation are also discussed in the first mechanism.

Following the suggestion of the reviewer, the three mechanisms have been clarified at the beginning of the section: 1) Increased synthesis of deleterious complex lipids; 2) Impaired function of cellular organelles; 3) Receptor-mediated inflammation.

As the reviewer indicates, inflammation and ER stress are also mentioned in the first mechanism because some of these pathways intersect. However, the increased synthesis of ceramide and diacylglycerol provokes additional effects, independent of ER stress or inflammation, which also lead to insulin resistance. For example, diacylglycerol and ceramide have the capacity to activate PKC. This kinase phosphorylates IRS1 at serine residues, attenuating the insulin signaling pathway.

Comment 4. Third section "Oleic acid prevents palmitic acid-induced insulin resistance in metabolic tissues" is short. Also, what is written in this section is already discussed in the first section. It is not really clear for the reader why it appears again.

This paragraph has been rephrased and the subheading has been eliminated. We have kept this paragraph as the introduction for the next subheadings of the manuscript.

Comment 5. Authors continue the paper by discussing the adipose tissue, skeletal muscle, liver and beta cells in separate sections. Such a design makes readers to think that the protective effect of oleate is tissue-specific. However, most mechanisms behind the toxic as well as protective effects of fatty acids are common for all tissues. For example, the role of GPR120 and inflammasome are discussed only in “adipose” section, which can make readers with no expertise in the field to think that this mechanism is adipose-specific.

We believe that the re-organization of the revised version of the manuscript has solved this problem (please, see comment 1).

Minor comments:

Comment 1. Some sentences can be formulated in a better way. Examples from page 3: "More than 90% of type 2 diabetic patients are overweight or obese, and obesity is associated with insulin resistance”; "Plasma NEFA arise from adipose tissue and the FA content of this tissue reflects fat intake”; "Although elevated plasma NEFA contribute to T2DM development, the saturated FA (SFA) (See Box 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differ significantly in their contribution to insulin resistance”.

Example 1: Following the suggestion of the editor, this sentence has been deleted in the revised version of the manuscript.
Example 2: This sentence has been substituted by “Plasma NEFA released by adipose tissue reflects fat intake”.
Example 3: This sentence has been substituted by “However, the saturated FA (SFA) (Box 1) palmitic acid and the monounsaturated FA (MUFA) oleic acid differently contribute to insulin resistance”.

**Comment 2.** In "adipose" section authors discuss about the diabetogenic effect of the tissue and the role of macrophages in this process but do not provide references.

Following the suggestion of the reviewer, we have included two additional references in this paragraph:


**Comment 3.** In the figures, palmitate uptake is shown to be mediated only by CD36 but this mechanism constitutes only 50% of the total uptake.

The reviewer is right. In the revised version of the manuscript we have included FATP.